

IN THE CLAIMS:

Sub B
1. (Amended) [Method] A method of [simultaneous] simultaneously screening for one or more gene insertion mutants in a population of any organism or cell line derived thereof, [by], said method comprising:

[a)] preparing an insertion element mutant library comprising a plurality of nucleic acid insertion elements and originating from a defined population of an organism [or cell line] wherein said [insertion(s)] gene insertion mutants [have] are to be detected[.];

[b)] amplifying [the] insertion element flanking sequences from said insertion element mutant library[.]; and [c1)] either

fixing [the] a set of [thus obtained] nucleic acid amplification products representing said insertion element flanking sequences derived from said insertion element mutant library to a solid support as target for hybridization, or

[c2)] producing a set of labelled amplification products representing said insertion element flanking sequences derived from said insertion element mutant library to use as probe to hybridize to a solid support to which one or more nucleic acids have been fixed as target(s) for hybridisation.

2. (Amended) [Method] The method according to claim 1 wherein the [thus obtained] set of nucleic acid amplification products [in step b)] representing said insertion element flanking sequences are obtained by iPCR using at least one primer or a set of primers based on [the] a sequence of [the] at least one insertion element.

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3. (Amended) [Method] The method according to claim 2 wherein the iPCR [is performed by] comprises:

[a)] digesting [the] nucleic acid sequences of said insertion element mutant library with [a] at least one restriction enzyme [which optionally recognizes motifs of four nucleotides in the genomic DNA, or with a combination of restriction enzymes] resulting in a collection of amplifiable genomic fragments[.];

[b)] self ligation of the] ligating at least one amplifiable genomic [fragments thus obtained]

fragment by self ligation; and either

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c1) [amplification of insertion element flanking sequences] amplifying said at least one amplifiable genomic fragment using a set of internal primers or
[c2) amplification of] amplifying insertion element flanking sequences using a [(set of)] a primer or set of primers based on the terminal part of the insertion element.

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4. (Amended) [Method] The method according to claim 3 wherein the insertion element flanking sequences are amplified by said set of internal primers, and the amplification products [of step c1] are re-amplified using at least one primer or a set of two nested primers based on [the]a sequence of the insertion element.

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5. (Amended) [Method] The method according to claim 1 wherein [the amplification products in step b) are obtained by] amplifying insertion element flanking sequences from said insertion element mutant library comprises amplifying said insertion element flanking sequences using transposon display amplification.

6. (Amended) The method [Method] according to claim 5 wherein said transposon display amplification [is performed by]comprises:

[a] generating at least one restriction fragment corresponding to each of said plurality of nucleic acid insertion elements by digesting [the]a plurality of nucleic acid sequences [of] included in said insertion element mutant library [with]using a first restriction enzyme that recognizes six conserved nucleotides [in the insertion element] and [with] a second restriction enzyme that recognizes a motif of four nucleotides [in the genome generating at least one restriction fragment per insertion containing at least the hexacutter site, a part of the insertion element, and part of the insertion element flanking sequence], said at least one restriction fragment including at least a tetracutter site, a hexacutter site, a part of an insertion element of said plurality of insertion elements, and at least part of an insertion element flanking sequence corresponding to said insertion element;

[b] ligation of] ligating a biotinylated adaptor to the hexacutter [sites and a ligation of]site of each of said at least one restriction fragment as well as a second adaptor to the tetracutter

[sites]site of said at least one restriction fragment [the restriction fragments generated in a,];
[c) selection of]selecting biotinylated restriction fragments using magnetic streptavidin beads[.];
[d) amplification of]amplifying insertion element flanking sequences using a primer based on [the]a sequence of the biotinylated adaptor and on the insertion element sequence and a primer complementary to the second adaptor[,]; and
[e) re-amplification of]re-amplifying said insertion element flanking sequences using a nested primer based on [the]an insertion element and a primer complementary to the second adaptor.

7. (Amended) [Method]The method according to [any of the preceeding claims]claim 1 wherein the solid support is a filter, micro-array, or chip containing nucleic acid sequences.

8. (Amended) [Method]The method according to [any of the preceeding claims] claim 6 wherein the nucleic acid sequences [is] are selected from a group consisting of genomic DNA [or] and cDNA.

9. (Amended) [Method]The method according to [any of the preceeding claims]claim 1 wherein preparing the insertion element mutant library comprises [of] preparing an insertion element mutant library including 30 DNA samples from 100 plants each.

10. (Amended) [Method]The method according to claim 9 wherein preparing the insertion element mutant library including 30 DNA samples from 100 plants each comprises preparing an insertion element mutant library [is] built in a 3D array of 10 Block, 10 Row and 10 Column pool each containing DNA of 100 plants characterised by the three coordinates B, R, C.

11. (Amended) [Method]The method according to [any of the claims 1-4] claim 3 wherein digesting nucleic acid sequences of said insertion element mutant library with at least one restriction enzyme comprises digesting nucleic acid sequences using BfaI [is used] as a restriction enzyme.

12. (Amended) [Method]The method according to [the claims] claim 5 [or 6] wherein amplifying said insertion element flanking sequences using transposon display amplification

comprises using a restriction enzyme selected from a group consisting of MseI and/[or] MunI [are used as restriction enzyme].

13. (Amended) [Kit] A kit for performing [any of the methods] the method of claim [1-12]1 comprising [at least] DNA samples of an insertion element mutant library [and optionally a set of restriction enzymes and/or primers].

14. (Amended) [Kit for performing any of the methods of claim 1-12] The kit of claim 13 further comprising [at least] a set of amplified insertion element flanking sequences.

15. (Amended) [Kit according to] The kit of claim 14 wherein the set of amplified insertion element flanking sequences have been fixed on a solid support, such as a filter, micro-array, or microchip, containing nucleic acid sequences.

Sub B3 16. (Amended) [Kit according to claims 14 or 15] The kit of claim 14 wherein the set of amplified insertion flanking sequences is [either] present in a state selected from a group consisting of a soluble [form or] state and a dried [form] state.

17. (Amended) [Kit according to] The kit of claim 16 wherein the set of amplified insertion element flanking sequences are labelled with [for instance] fluorescein.

Please add the following new claims:

Sub B4 18. A method for simultaneously screening one or more gene insertion mutants in a cell line comprising:

a 2 preparing an insertion element mutant library comprising a plurality of nucleic acid insertion elements and originating from a cell line wherein said gene insertion mutants are to be detected;

amplifying insertion element flanking sequences from said insertion element mutant library; and fixing a set of nucleic acid amplification products representing said insertion element flanking